THE DEVELOPMENT OF THE OVULE AND THE SEED IN THE HYPOXIDEAE.

PART I.

IANTHE SALISB.

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This investigation was undertaken to compare the development of the ovule and the seed in the genus Ianthe (Hypoxis L.) with that in Pauridia Harv. After the work was under way, Stenar's publication (1925) on the embryology of two species of Hypoxis was received. It became evident that in *Ianthe aquatica* the formation of the endosperm differs from that in the species investigated by Stenar, and it was therefore considered necessary to examine more species of Ianthe to determine the type of endosperm development in other South African representatives of the genus.

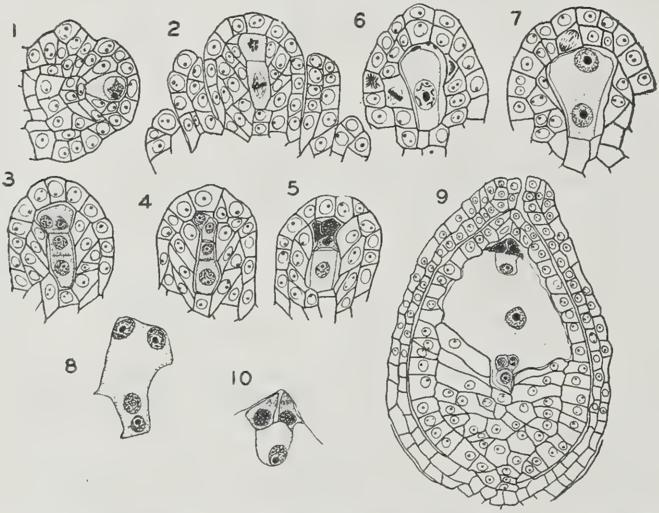
Method.—The plants were collected on the Stellenbosch Flats. Ovaries in different stages of development were fixed in the FAA fixative and after embedding were cut 8 to 12 μ in thickness and treated either with Heidenhain's iron-alum haematoxylin or with Delafield's haematoxylin, the former to show the nucleus and cell divisions, and the latter to show up the cell-walls. Microchemical tests were made to determine the character of the cell-walls. As tests for reserve food-materials in the endosperm had already been made (Markötter, 1936), they were not repeated.

Ianthe aquatica (L.f.) Williams.

The anatropous ovules possess two integuments and are arranged in two rows in axile placentations.

Gametogenesis.—The single-celled archesporium is hypodermal in origin and functions directly as a megaspore mother-cell. It enlarges considerably before undergoing reduction division. In the first meiotic division the spindle lies parallel to the longitudinal axis of the ovule, and during the interphase a cell-wall is formed between the two daughter nuclei. In the second reduction division the spindle in the lower cell again lies parallel to the longitudinal axis, but the one in the upper cell is placed more or less at right angles to it. After this division two other cell-walls are laid down in such a way that the four megaspores show a T-shaped arrangement. Sometimes they are in a nearly linear position, the upper one, however, being slightly out of the straight line.

Sometimes two megaspore mother-cells lying next to each other are formed in one ovule, and in one case it was found that one of these mother-cells had degenerated. A few ovules contained two perfectly normal embryo-sacs separated from each other by a layer of nucellar cells, and this shows that two megaspore mother-cells can develop in one ovule.



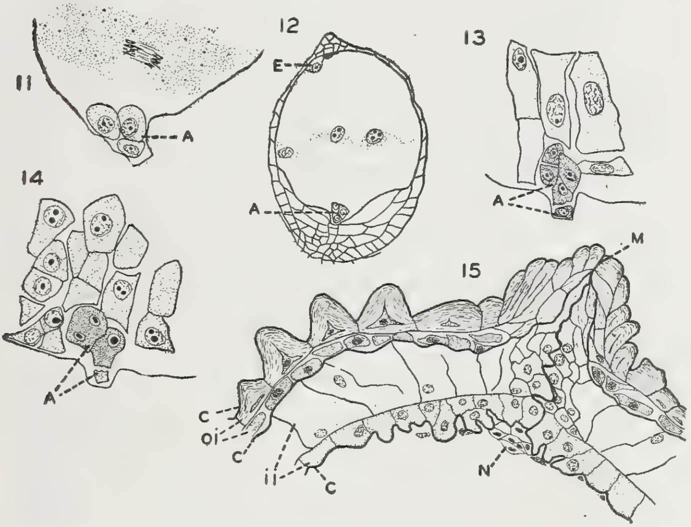
Figs. 1—10. Formation of megagametophyte in *Ianthe aquatica*. 1. Megaspore mother-cell; 2. Second reduction division of the megaspore mother-cell; 3. Cytokinesis after the second reduction division; 4. Four megaspores; 5. Functioning megaspore, with the others degenerating; 6. Functioning megaspore enlarging, the other megaspores having been absorbed; the nucellus has divided to form two cell layers above the megaspore; 7. Young embryo-sac with two nuclei; 8. Young embryo-sac with four nuclei; 9. Mature embryo-sac; the outer integument is not shown; 10. Egg-apparatus of the embryo-sac, showing the filiform apparatus of the synergids.

Figs. 1—8 and 10 × 450; Fig. 9 × 300.

The nucellus is small. At the micropylar end the megaspores are covered only by the epidermis of the nucellus, but when the functioning megaspore enlarges, a few of these epidermal cells divide by periclinal walls to form a two-layered nucellar cap.

After three nuclear divisions the megaspore at the chalazal end develops into a normal eight-nucleate embryo-sac, while the other megaspores degenerate and are absorbed. The basal end of the embryo-sac remains narrow, but the upper part widens considerably after it has absorbed some of the nucellar cells along its sides. Here the embryo-sac is covered only by the nucellar epidermis, which is very rich in protoplasmic contents and differs in this respect from all other cells in the ovule. At the base of the embryo-sac the nucellus is pluri-layered.

The embryo-sac has three antipodal cells crowded into the narrow basal end, and at the opposite end an egg-apparatus consisting of the egg-cell and the two synergids. The latter show a " filiform apparatus " in their pointed ends. Before the flower opens the two polar nuclei fuse to form the secondary nucleus, which lies in the centre of the embryo-sac, attached to its sides by strands of protoplasm.



Figs. 11—15. Formation of endosperm and testa in *Ianthe aquatica.* 11. First division of the primary endosperm nucleus ; A. Antipodal cells ; 12. Embryo-sac with a few free endosperm nuclei ; E. Fertilised egg-cell ; 13. Persistent antipodal cells, two having undergone cell division ; endosperm cells not yet filling the embryo-sac cavity ; 14. Persistent antipodals, one with two nuclei, in older endosperm tissue ; 15. Nearly mature testa ; C. Cuticle ; II. Inner integument ; OI. Outer integument ; M. Old micropyle ; N. Nucellar cap. Fig. 11 × 450 ; Fig. 12 × 150 ; Figs. 13—15 × 300.

Fertilisation and Endosperm.—The pollen tube enters through the micropyle, and soon after fertilisation has taken place, the primary endosperm nucleus begins to divide. The spindle of the first nuclear division is in a transverse position. By repeated divisions large numbers (200 to

300 nuclei were counted) of free nuclei are formed, which are arranged in a single layer within a band of cytoplasm along the walls of the embryo-sac. The centre of the sac is filled with cell-sap.

After the young embryo has reached the 32-celled stage, cell-walls are laid down between all the endosperm nuclei, thus forming a layer of cells, which increase in number by repeated cell divisions and later completely fill the embryo-sac with endosperm tissue.

No basal cell is cut off from the primary endosperm nucleus, as Stenar found in *Hypoxis decumbens* (1925).

In a large number of ovules, especially those in the base of the ovary, hypertrophy of the endosperm nuclei occurs before the cell-walls are formed. Such embryo-sacs contain a small number of large lobed nuclei with large nucleoli and chromatin lumps. Some of these nuclei are 120 by 20 μ in size, and a few were seen to divide by multipolar spindles. The embryo in such ovules begins to develop, but later has a shrunken appearance, and these ovules probably degenerate.

The Antipodal Cells.—The antipodals undergo a varying fate. In a number of ovules they degenerate soon after the fertilisation. In most cases, however, they live for some time after the fertilisation and may even persist until the endosperm fills the embryo-sac completely. One or two of them may increase in size, and in a small number of cases the nucleus divides, and this may be followed by a division of the cell.

Due to the well-developed integuments, a very narrow chalazal passage is left through which the nutritive material has to pass to the embryo-sac, and some greyish droplets visible in the base of the endosperm cavity indicate that materials enter the embryo-sac through this passage.

The Embryo.—After the primary endosperm nucleus has formed a number of nuclei, the fertilised egg-cell, hanging from the nucellar cap into the cavity of the embryo-sac, begins to divide. The first division is by a transverse wall into an apical and a basal cell, *ca* and *cb*. The apical cell divides by a longitudinal wall slightly before the division of the basal cell, which divides by a transverse wall into *m* and *ci*. This is the quadrant stage.

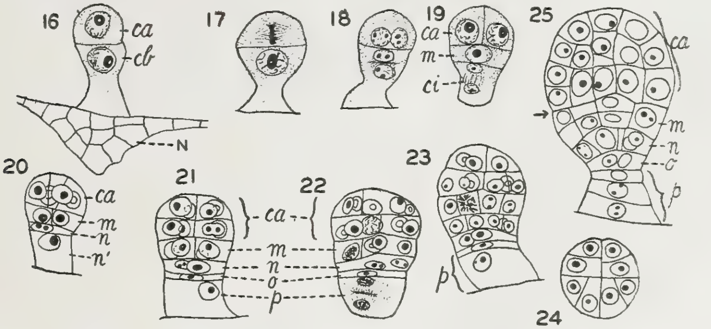
The new basal cell *ci* again divides by a transverse wall into *n* and *n′*, the two cells of *ca* divide by another longitudinal wall at right angles to the first to form four cells, and in the middle cell *m* a longitudinal wall is laid down. In this way the octant stage is reached, *ca* and *cb* each having contributed four cells.

The basal cell *n′* divides by a transverse wall into *o* and *p*, *n* by a longitudinal wall into two cells, the four cells of *ca* by transverse walls to form two layers of four cells each, and the two cells of *m* by longitudinal walls to form four cells. This gives the 16-celled stage to which *ca* and *cb* each contribute eight cells.

The new basal cell *p* divides by a transverse wall, both layers of cells of *ca* and the four cells of *m* by longitudinal walls into eight cells each, the two cells of *n* by longitudinal walls into four cells, and the cell *o* similarly into two cells. Thus the 32-celled stage is reached, 16 cells having originated from *ca* and 16 from *cb*.

(The cell divisions which bring the embryo from one stage to the next, do not take place simultaneously, nor in the order enumerated in each paragraph.)

The next cell divisions in the region of *ca* are more or less irregular. The dermatogen now becomes visible for the first time, but no periblem or plerome is differentiated. The embryo is formed not only from the apical cell of the two-celled pro-embryo, but the basal cell also contributes to its structure. The cells derived from *ca* form only the terminal cotyledon, and the hypocotyl and the radicle are formed by the layers *m*, *n* and *o*, derived from the basal cell. The suspensor is derived only from the cell *p*, which divides a few times by transverse walls to form a short row of cells.



FIGS. 16—25. Formation of embryo in *Ianthe aquatica*. 16. Two-celled pro-embryo; N. Nucellar cap; 17. Division of the apical cell, nucleus of the basal cell in prophase; 18. Cytokinesis before the quadrant stage; 19. Division of the basal cell *ci* into *n* and *n′*; 20. Octant stage; 21. Sixteen-celled stage; 22, 23. Some cell divisions to form the 32-celled stage; 24. Transverse section of 32-celled embryo, showing the second layer of *ca* with eight cells; 25. Undifferentiated embryo of nearly ripe seed; *p*, suspensor; *ca* will form the terminal cotyledon; position of growing point of plumule indicated by arrow. × 450.

The future growing point of the plumule is indicated on one side of the embryo by a small indentation where the cells in the dermatogen and adjacent layers divide faster than on the other side. Except for this slight indentation, the embryo of the mature seed is undifferentiated, and

Markötter, who investigated the germination, found that the seed has to undergo a long after-ripening process while lying on the ground during the winter. The embryo lies embedded in the endosperm and is attached to the small nucellar cap by means of a short suspensor.

The Integuments and Testa.—The integuments begin to develop before the megaspore mother-cell undergoes reduction division, and at the time of fertilisation they enclose the nucellus completely. The outer integument is not reduced near the funiculus. The micropylar canal is formed by both integuments, but the endostome is longer than the exostome. Each integument consists of two layers of cells, except at their extreme tips, where they are three-layered. Their surfaces as well as that of the nucellus are covered by a thin cuticle which hardly thickens in the ripening seed.

The testa of the ripe seed is formed by the two integuments, both of which largely remain two-layered. Here and there a few cells of the second layer of the outer integument divide by periclinal walls. At the chalaza and along the raphe, however, the seed-coat consists of more than four layers of cells.

The Outer Integument.—An outgrowth is produced from the external wall of each cell of the outer layer, giving the testa a warty appearance. This outer cell-wall becomes thickened to such an extent that the lumina of the cells are completely obliterated. Schultz's solution shows it to consist of cellulose. No lignin or suberin is present, but later this layer turns yellow and then black due to the impregnation of the cellulose layers with some other substance. The outer cuticle covering this layer is only slightly thickened.

In the nearly ripe seed the second layer of cells is still rich in protoplasmic contents and the nuclei are visible. The cells are flat and remain thin-walled and contain some starch grains. The cuticles separating the two integuments fuse to form one layer.

The Inner Integument.—The cells of the outer layer enlarge considerably and are very poor in protoplasmic contents. A few nuclei are still visible when the seed is nearly ripe, but later these cells disintegrate by the breaking-down of some of the anticlinal walls. This layer probably becomes filled with air, and in the ripe seed it is compressed to a small part of its former width, due to the pressure exerted by the developing endosperm. In the nearly ripe seed the inner layer of cells still retains its protoplasmic contents, and the cells are large but flat, except at the micropyle where they are quite small. The nucellar cuticle fuses with that on the inner surface of this integument, and in the micropylar region deep folds are observed in it. The old endostome canal is likewise lined with a deeply-folded cuticle.

The Nucellus is completely absorbed except for the cuticle, a few cells at the base and the small nucellar cap at the micropyle.

Ianthe alba (L.f.) Salisb., **I. minuta** (L.f.) Williams and **I. Schlechteri** (Bol.) Williams.

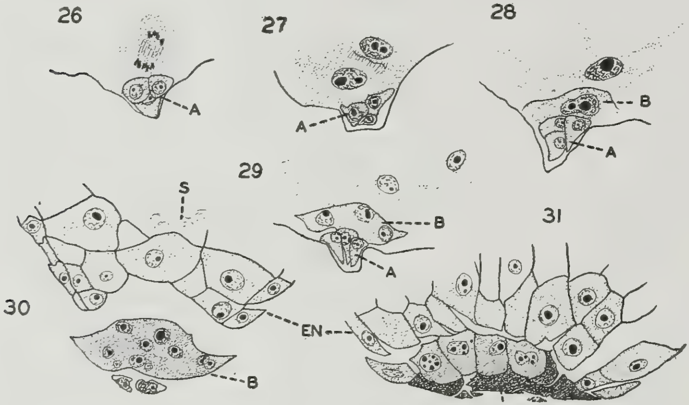
Gametogenesis.—The structure of the ovule and the development of the female gametophyte in these species are identical with that of *I. aquatica.* The ovules are anatropous and possess two integuments. The megaspore mother-cell is hypodermal and forms a T-shaped row of megasspores, the basal one of which develops into a normal eight-nucleate embryo-sac, which is covered only by the nucellar epidermis, except for the two- or three-layered nucellar cap.

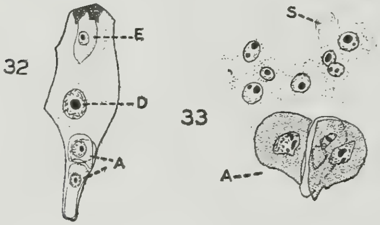
Endosperm and Antipodal Cells.—In *I. alba* and *I. minuta* the development of the endosperm is nuclear and is similar to that of *I. aquatica.* The antipodal cells persist for some time after the fertilisation. In *I. alba* they degenerate when the embryo has reached the quadrant to octant stage and before cell-walls are laid down among the free endosperm nuclei. In *I. minuta* they grow larger than in *I. aquatica* and their nuclei possess large chromocentres and resemble those of glandular cells. Sometimes one of the nuclei divides. In some ovules they begin to degenerate when there are sixteen endosperm nuclei present, but in other cases they persist until cytokinesis takes place in the endosperm. After their degeneration a number of the adjacent endosperm cells becomes filled with a densely granular cytoplasm. Greyish droplets of some substance are visible opposite the antipodals in the base of the endosperm cavity.

In *I. Schlechteri* helobial endosperm formation takes place. The primary endosperm nucleus which lies near the antipodals, divides with a longitudinal spindle, thus forming two nuclei situated one above the other. The lower nucleus, lying next to the antipodal cells, is cut off from the upper one by a cell-wall or membrane, and in this way a basal endosperm cell is formed. The upper nucleus forms the endosperm tissue, first by repeated nuclear divisions until there are about 300 free nuclei present, arranged in a layer on the periphery of the embryo-sac, then by the simultaneous formation of cell-walls, and later by normal cell division.

In the basal endosperm cell the nucleus also divides repeatedly and forms a multinuclear chamber with dense cytoplasm and at least 16 nuclei, crowded together. Afterwards this chamber divides irregularly into a number of cells, a few of which have a single nucleus and others more than one. No granular food-reserves are stored in these cells, and later they become flattened and subsequently degenerate. In the endosperm cavity some greyish droplets are visible opposite the basal cell.

The nuclear divisions in the upper and lower chambers do not occur simultaneously, the mitoses in the upper chamber or endosperm proper being more frequent.





Figs. 26—31. Formation of endosperm in *Ianthe Schlechteri*. 26. First division of primary endosperm nucleus ; 27. A cell-wall or membrane being formed after the first division of the primary endosperm nucleus ; 28. Embryo-sac divided into a basal and an upper chamber, each with one nucleus ; 29. Basal endosperm cell showing three out of four nuclei, upper cell with two free nuclei ; 30. Basal cell with a number of nuclei ; endosperm tissue in the upper chamber ; 31. Older endosperm tissue ; the basal cell has divided into a number of cells, some of which are degenerating ; A. Antipodal cells ; B. Basal endosperm cell ; EN. Endosperm tissue ; S. Droplets. Figs. 26—28 × 450 ; Figs. 29—31 × 300.

Figs. 32 and 33. *Ianthe minuta*. 32. Mature embryo-sac after fertilisation ; 33. Base of old embryo-sac showing free endosperm nuclei and persistent antipoda cells. A. Antipodals ; D. Primary endosperm nucleus ; E. Fertilised egg-cell ; S. Droplets. × 300.

When the basal endosperm cell has two or four nuclei, the antipodal cells degenerate, but they are still visible for some time as blackened masses. Hypertrophy of the endosperm is of rare occurrence in these three species.

Embryo.—The early stages in the development of the embryo in *I. alba* and *I. Schlechteri* are similar to those of *I. aquatica*. After the sixteen-celled stage the embryos were not studied except in the ripe seeds, where they were found to resemble those of the previously studied species.

Testa.—The structure of the testa in the three species under discussion is fundamentally similar to that of *I. aquatica,* and only slight differences occur between the species, *e.g.* the number and size of the cells of the outer layer and the length of the protruding outgrowths. In *I. minuta* these cells and protuberances are few in number and much larger than in *I. aquatica* and *I. alba*.

Another slight difference is found in the width of the outer integument. In *I. minuta* this consists of two layers of cells, but in *I. alba* the inner layer of this integument has been duplicated in the region of the raphe. In *I. Schlechteri* this integument is similar to that of *I. aquatica* and also is largely two-layered.

As in *I. aquatica,* the nucellus is absorbed in the ripe seed, except for the cuticle, the nucellar cap at the micropyle and some cells at the base of the endosperm.

DISCUSSION.

The development of the embryo in the species of Ianthe conforms to the Asterad type (Schnarf, 1929 ; Johansen, 1945). In this type the apical cell of the two-celled pro-embryo divides by means of a longitudinal wall, and both the apical and the basal cell contribute largely to the structure of the embryo.

The early development of the embryo is similar to that of *Anthericum ramosum* (Souèges, 1918). The latter found that in this species the cotyledon was derived from the apical cell, the hypocotyl from *m*, the hypophysis from *n*, and that *o* and *p* formed the suspensor. (The same symbols that Souèges employed are used in this investigation.) In Ianthe the apical cell of the pro-embryo also forms the cotyledon, and the other parts of the embryo are likewise formed from cells contributed by the basal cell. It was, however, impossible to determine the origin of the hypocotyl and the radicle, but it was evident that the suspensor in *Ianthe aquatica* is derived only from the cell *p* and that the cell *o* also contributes to the structure of the radicle.

The development of the ovule and the embryo-sac in the four species of Ianthe agrees with Stenar's results in *Hypoxis decumbens* and *H. villosa*, and this confirms the very close relationship between the two genera. The only difference was found in the formation of the endosperm and in the persistence of the antipodal cells. In the abovementioned species Stenar found the endosperm to develop according to the Helobiae type (1925 ; 1938, p. 281), but the present investigation shows that this occurs only in *Ianthe Schlechteri*, the other three species showing a nuclear endosperm formation. Ianthe is therefore another genus with two types of endosperm development. The formation of the endosperm therefore has no great systematic value, at least in the Hypoxideae, to show relationships with other genera. Whether it has any taxonomic importance within the genus, is impossible to say as yet, as the endosperm formation in only four out of about twenty species has been studied. These four species belong to the following subgenera (Nel, 1914*b*) :—

I. alba, *I. aquatica*, with 3 other species ..	Aquaticae
I. Schlechteri (the only species)	Pectinatae
I. minuta (the only species)	Minutae

The Aquaticae is the oldest group (Nel, 1914*a*) and the other two groups are more specialised, the Pectinatae being the most highly evolved. Only the latter shows endosperm of the helobial type.

In the three species with nuclear endosperm development the antipodal cells are large and persist for a long time after fertilisation, whereas in *I. Schlechteri* they degenerate earlier, but not before the fertilisation as Stenar found for the two species of Hypoxis. These persistent antipodal cells probably help in passing food-materials into the embryo-sac. The integuments are very deeply cut and all the materials that enter the embryo-sac have to pass through a very narrow passage opposite which these cells are situated. In *I. Schlechteri* this function of the antipodal cells is probably taken over by the basal endosperm chamber. That food materials enter the sac through the chalazal passage, is also indicated by greyish droplets visible opposite the passage in the endosperm cavity.

The antipodal cells and the basal endosperm chamber in *I. Schlechteri* do not enlarge to such an extent that they can be designated as typical chalazal haustoria, as Schlimbach (1924) found to be present in Curculigo.

Summary.

1. The development of the ovule and seed in the four species of Ianthe investigated is similar, except for the formation of the endosperm.

2. The hypodermal archesporial cell functions as megaspore mother-cell and forms a T-shaped row of megaspores, the chalazal one developing into a normal eight-nucleate embryo-sac. The nucellus remains small and the mature embryo-sac is covered only by the nucellar epidermis which is absorbed before the seed is ripe.

3. The antipodal cells persist for some time after fertilisation and in one species one or two of them may divide.

4. In the three species, *I. alba*, *I. aquatica* and *I. minuta* the development of the endosperm is nuclear, but in *I. Schlechteri* according to the helobial type.

5. The development of the embryo in *I. aquatica* is explained. It conforms to the Asterad type, and the apical cell of the two-celled proembryo forms only the terminal cotyledon.

6. The seed-coat is formed by the two integuments, and its structure is explained.

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